Cell sorting to screen for recombinant
enzymes
excise λ library into E.coli

recombinant E.coli

add enzyme substrate

recombinant enzyme
cleaves substrate
to make cells fluorescent

sort

isolate expression clone

FIG. 1

FIG. 2

Co-encapsulation   Growth and expression   Receptor binding of small
Library + Eukaryote   of small molecule from host   molecule & GFP reporting

E=Eukaryotic assay organism   L=Large insert library   SM=Small molecule
GFP= Green fluorescent protein   R=Eukaryotic receptor
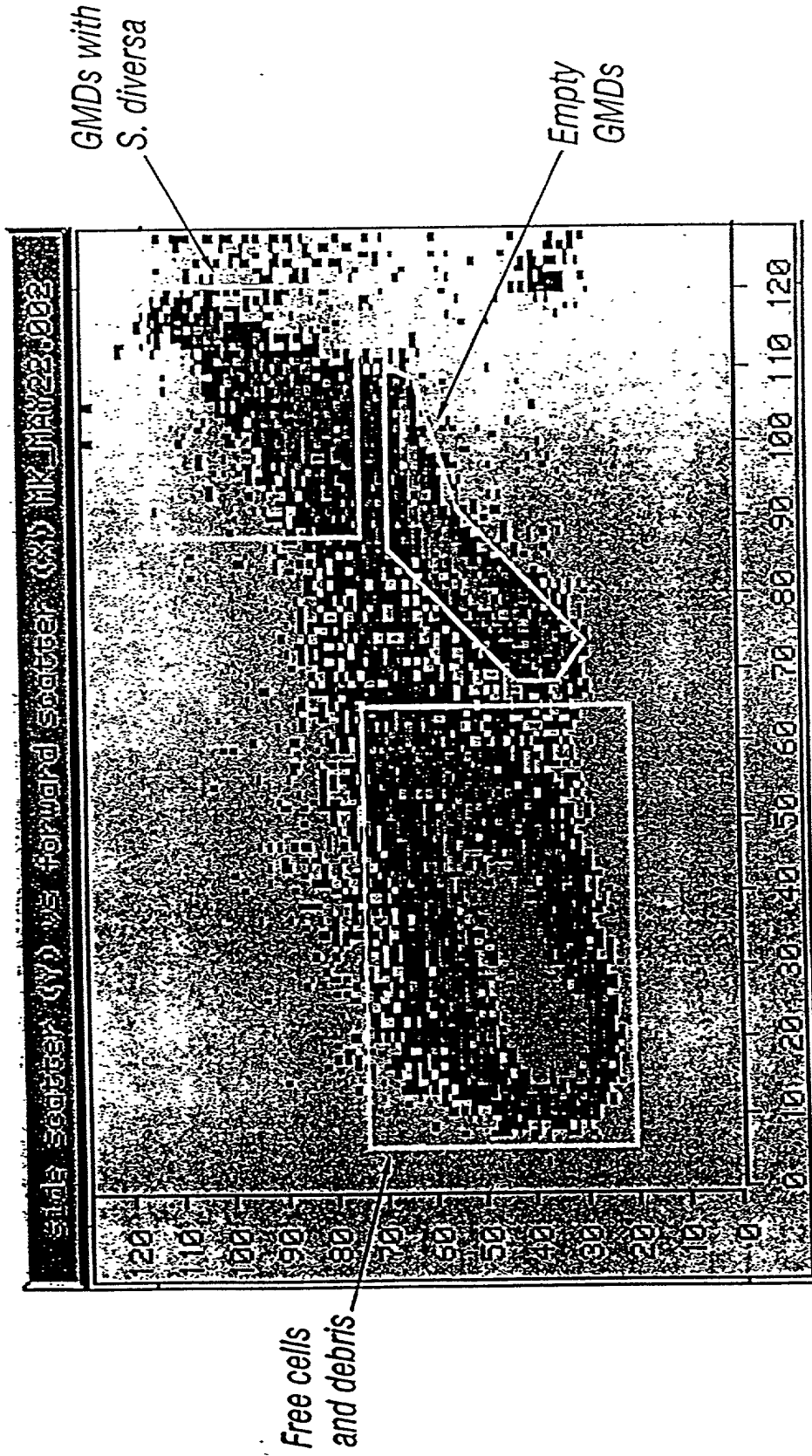
FIG. 3

FIG. 4

FIG. 5

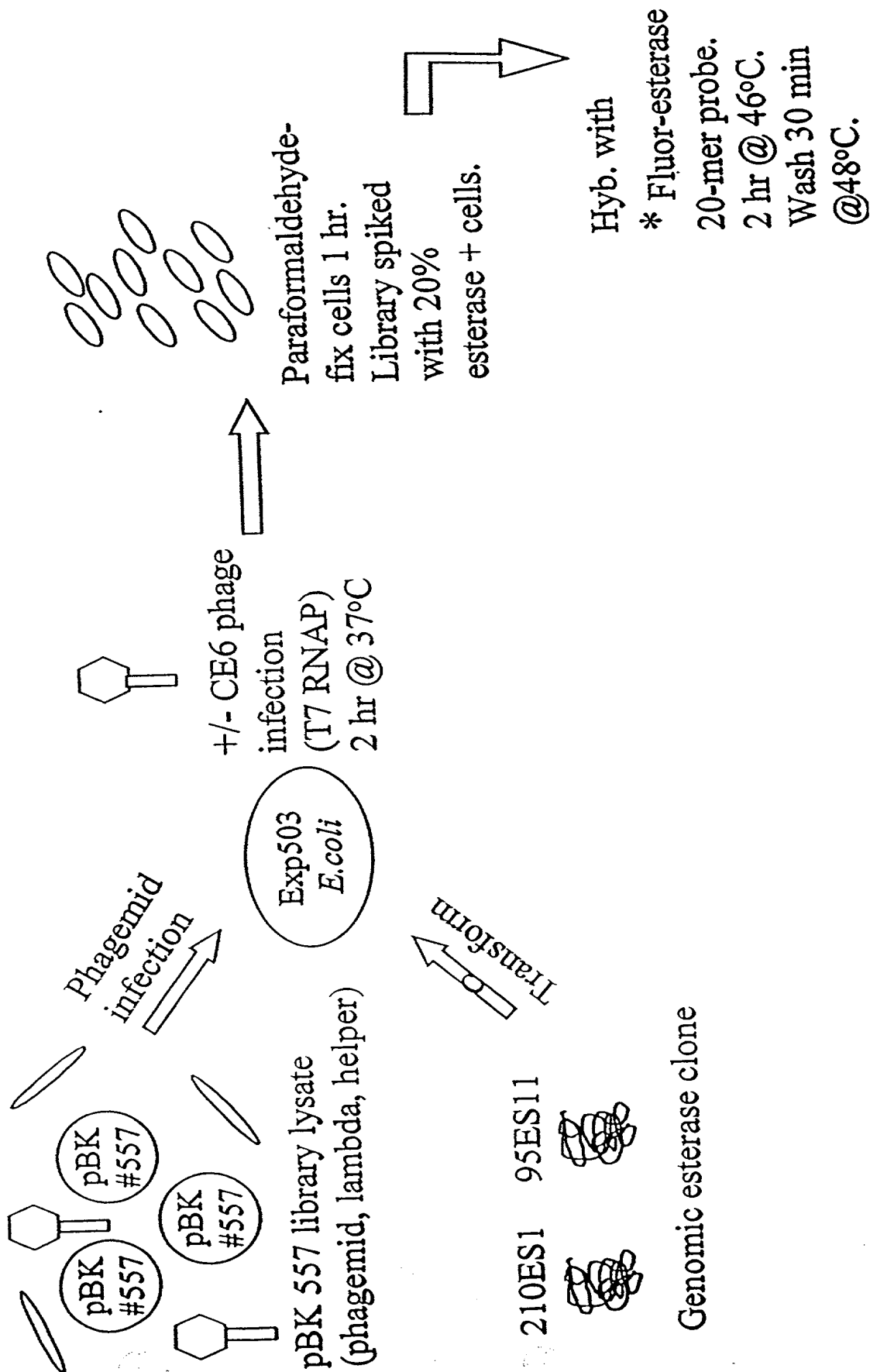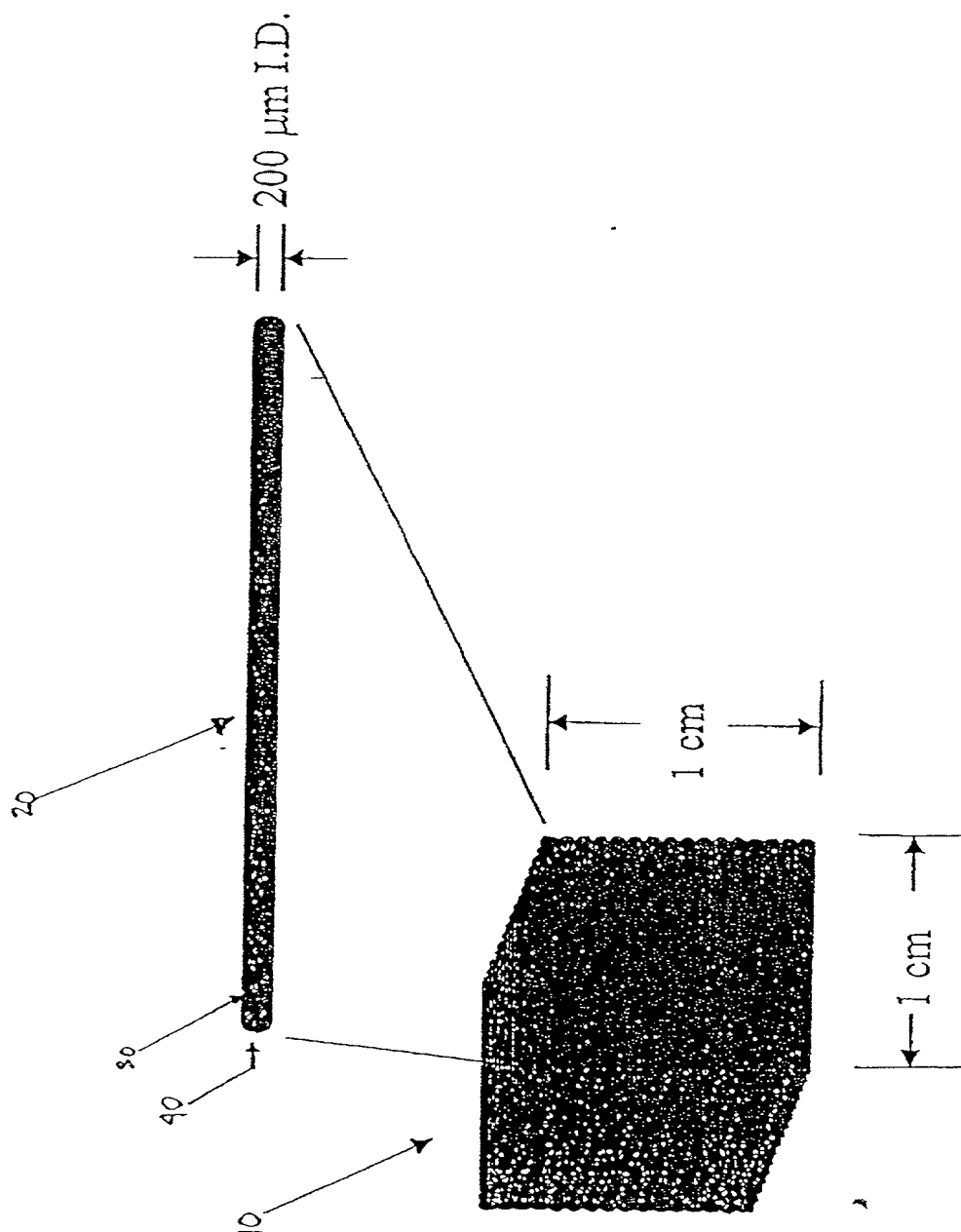# Whole Cell Hybridization Protocol
(+ control)

pBK #557
pBK #557
pBK #557

pBK 557 library lysate
(phagemid, lambda, helper)

Phagemid infection

210ES1 95ES11

Genomic esterase clone

Transform

Exp503
E.coli

+/- CE6 phage
infection
(T7 RNAP)
2 hr @ 37°C

Paraformaldehyde-
fix cells 1 hr.
Library spiked
with 20%
esterase + cells.

Hyb. with
* Fluor-esterase
20-mer probe.
2 hr @ 46°C.
Wash 30 min
@48°C.

200 µm I.D.

20

50

40

1 cm

1 cm

10
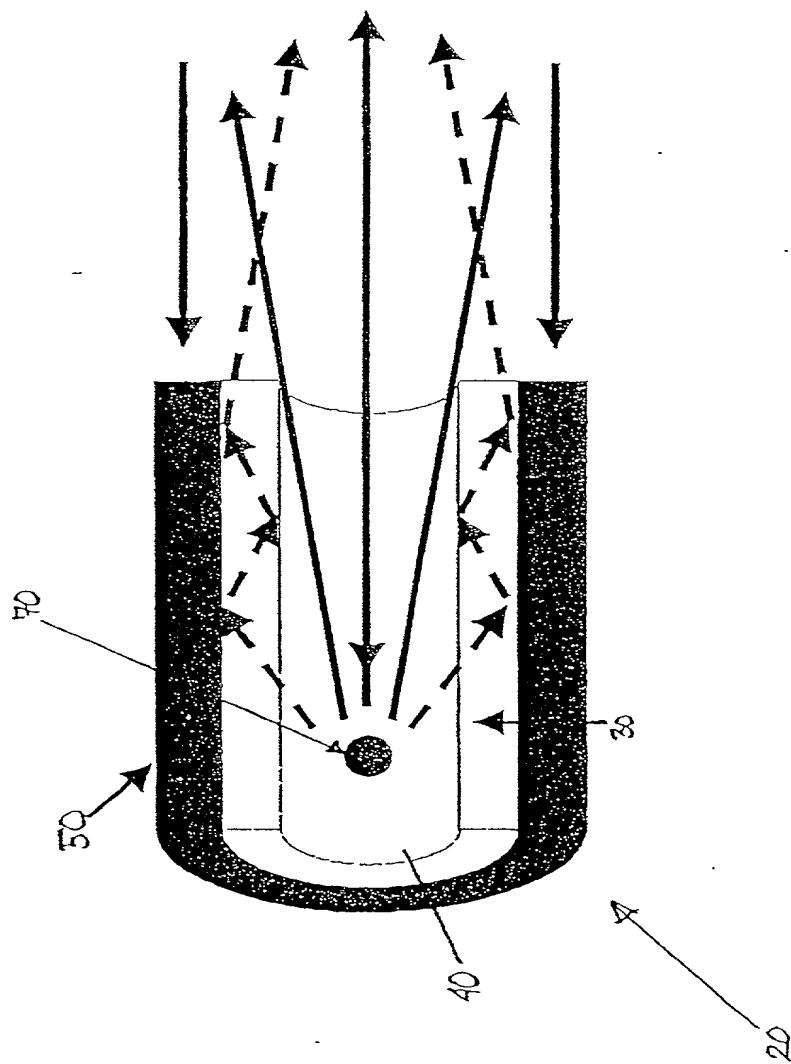
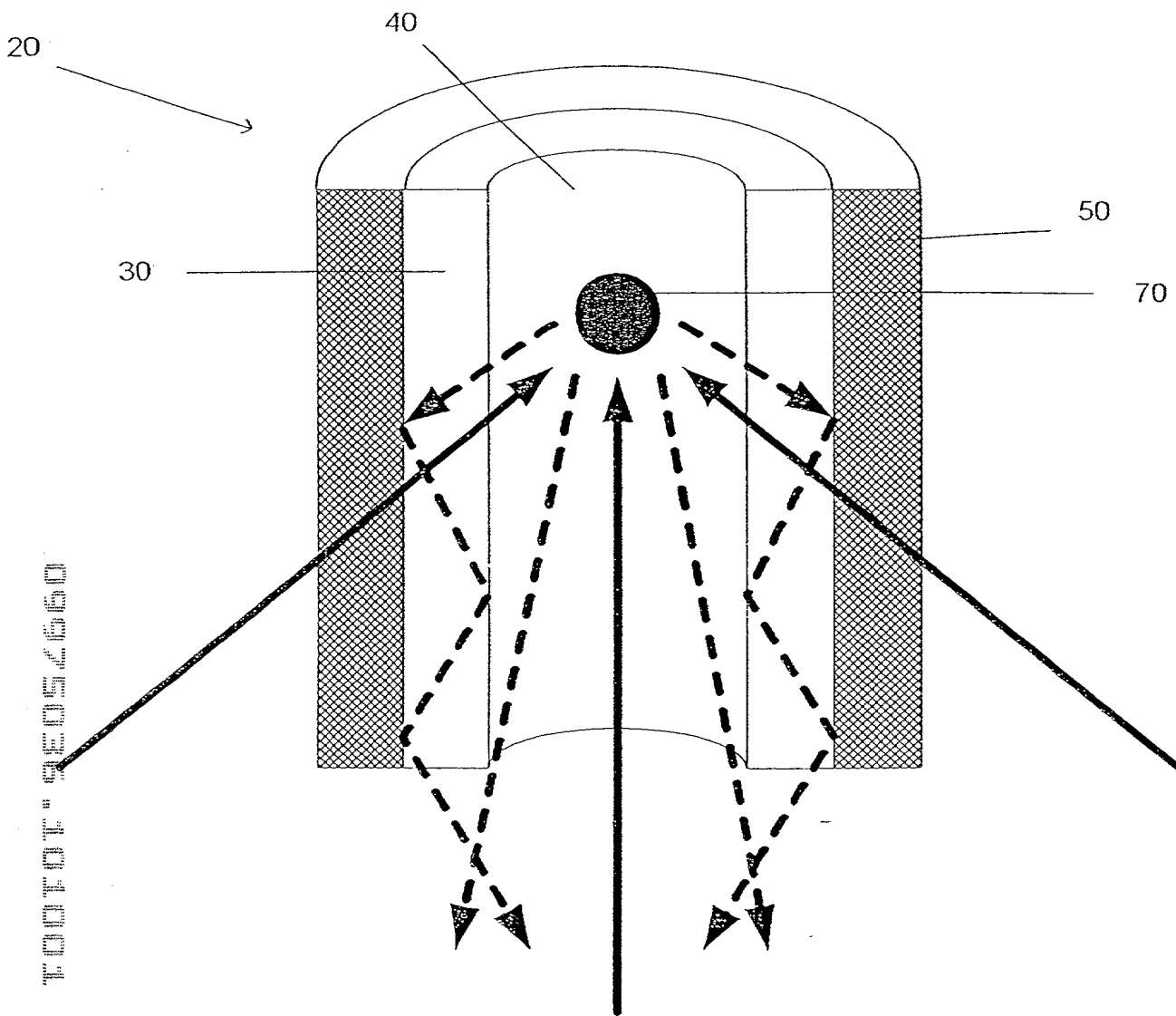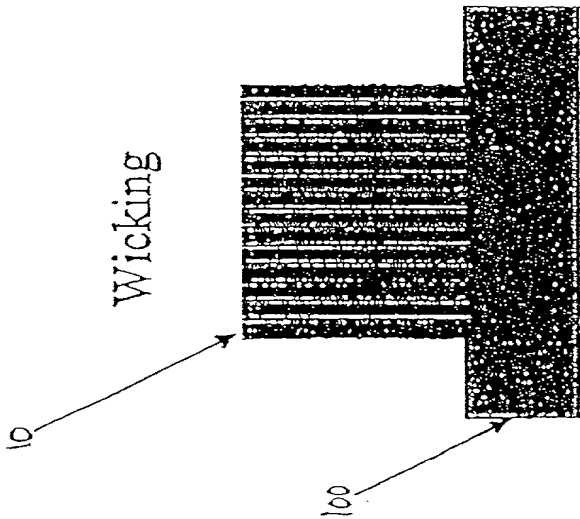Figure 6A

Figure 6B

FOOTPRINT: 0.60"x0.60"
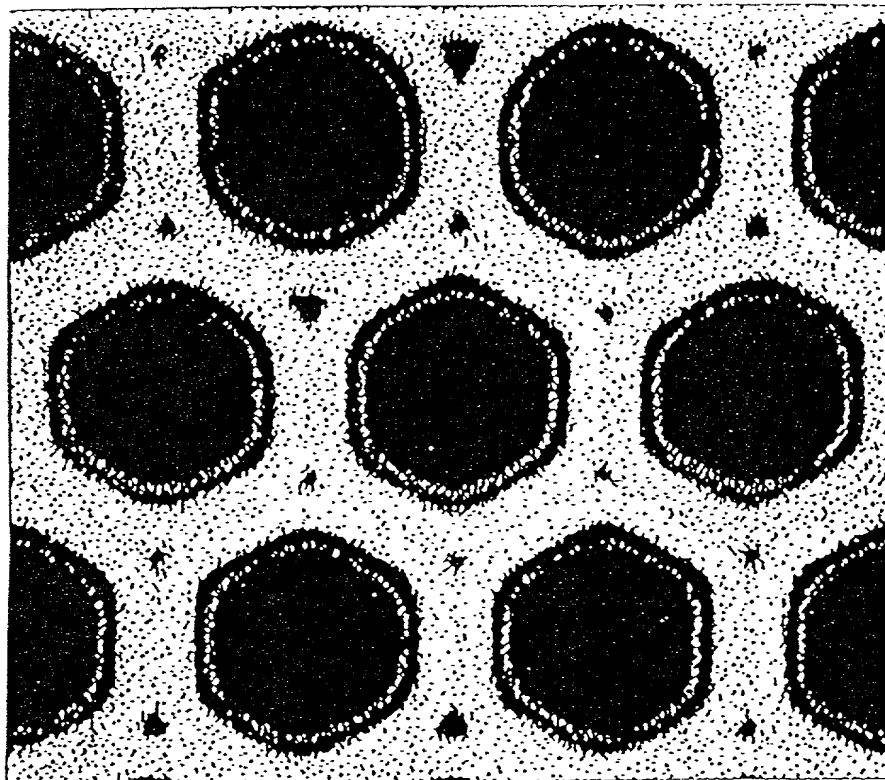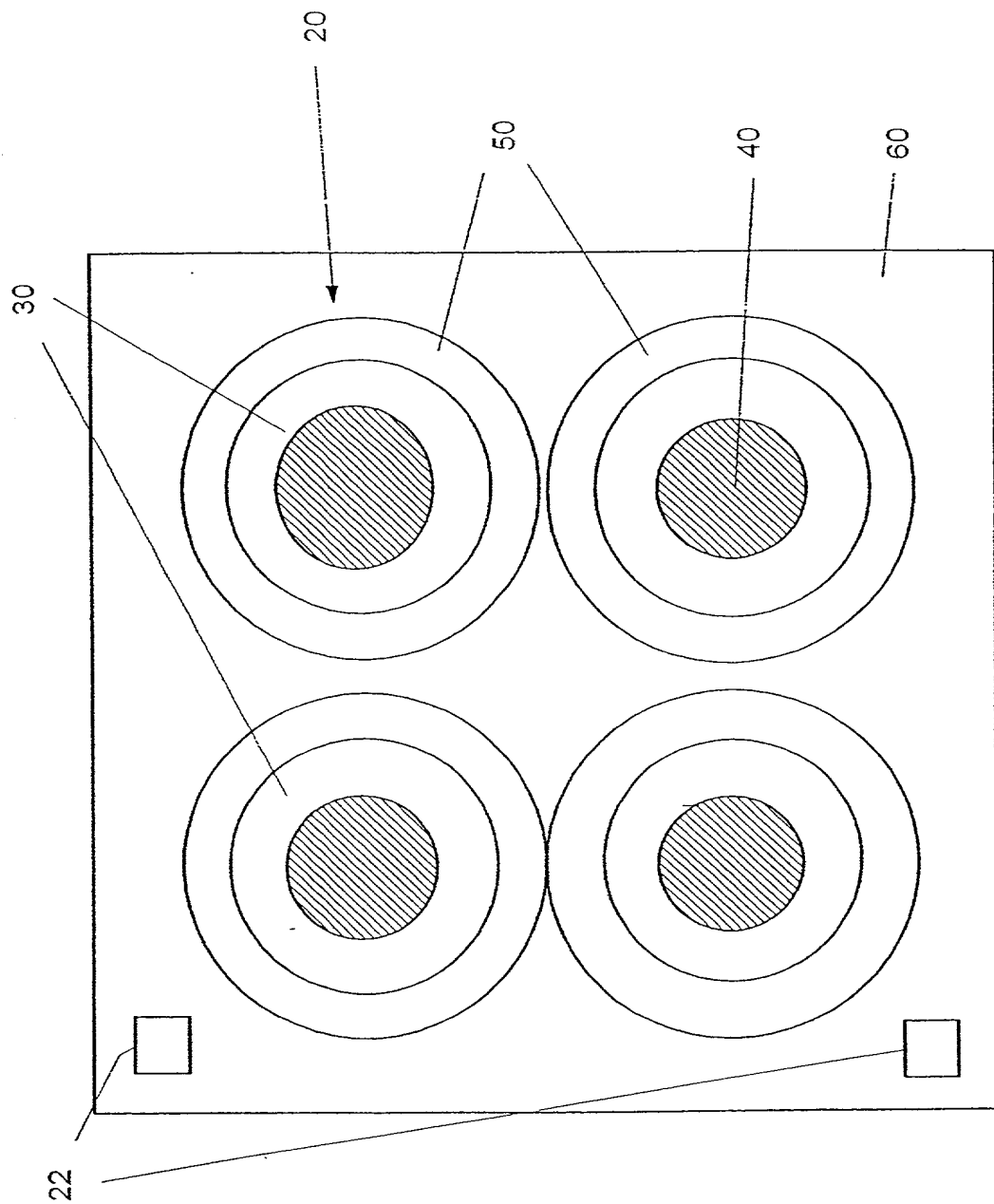
FIG. 7

Figure 8

Figure
9

Wicking   Humidified Incubation   Imaging & Recovery
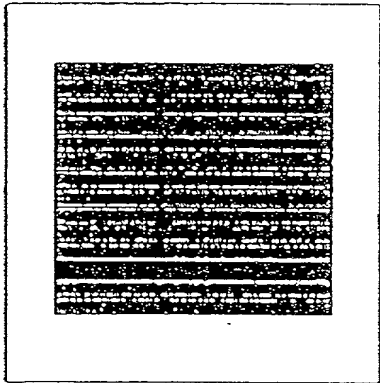
Figure 10
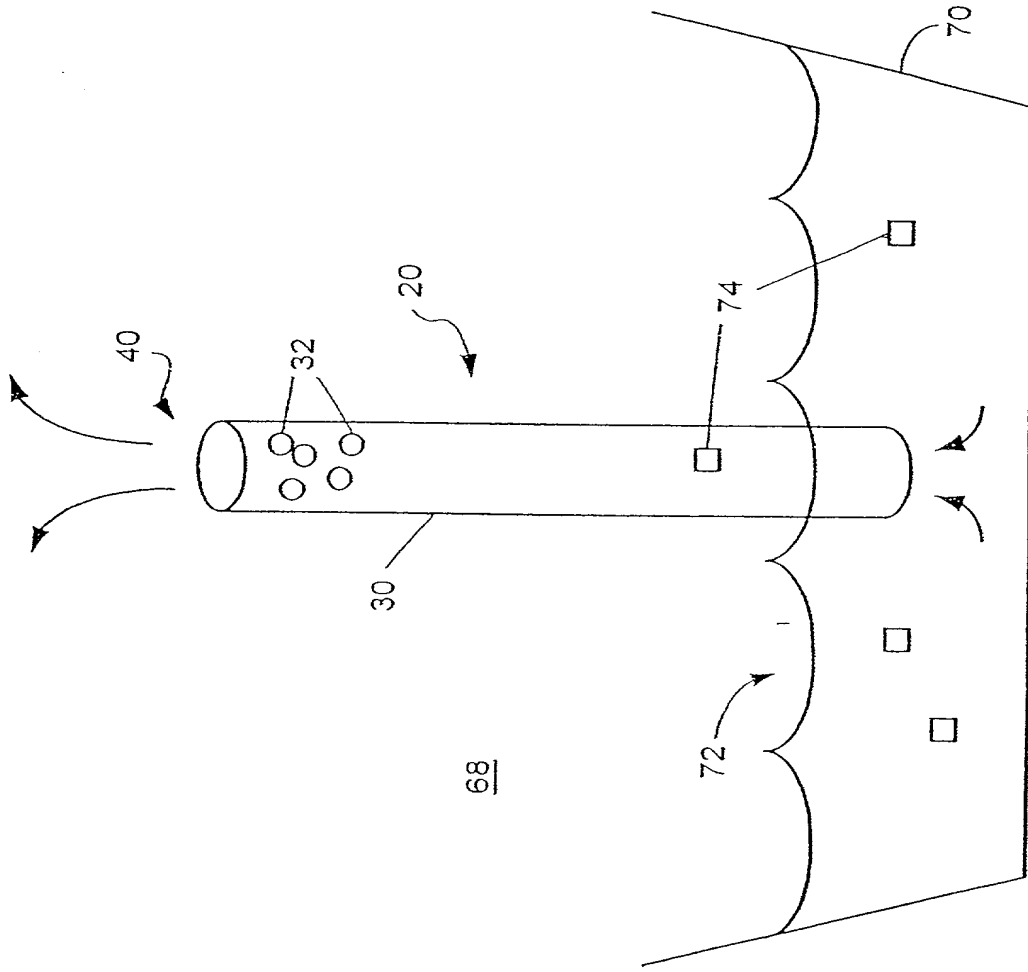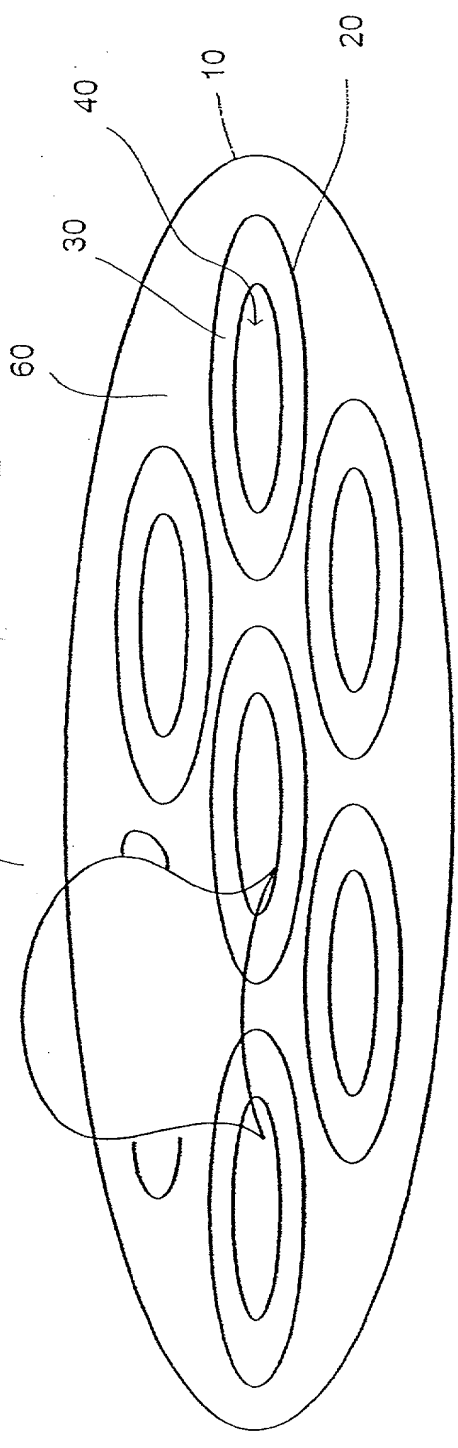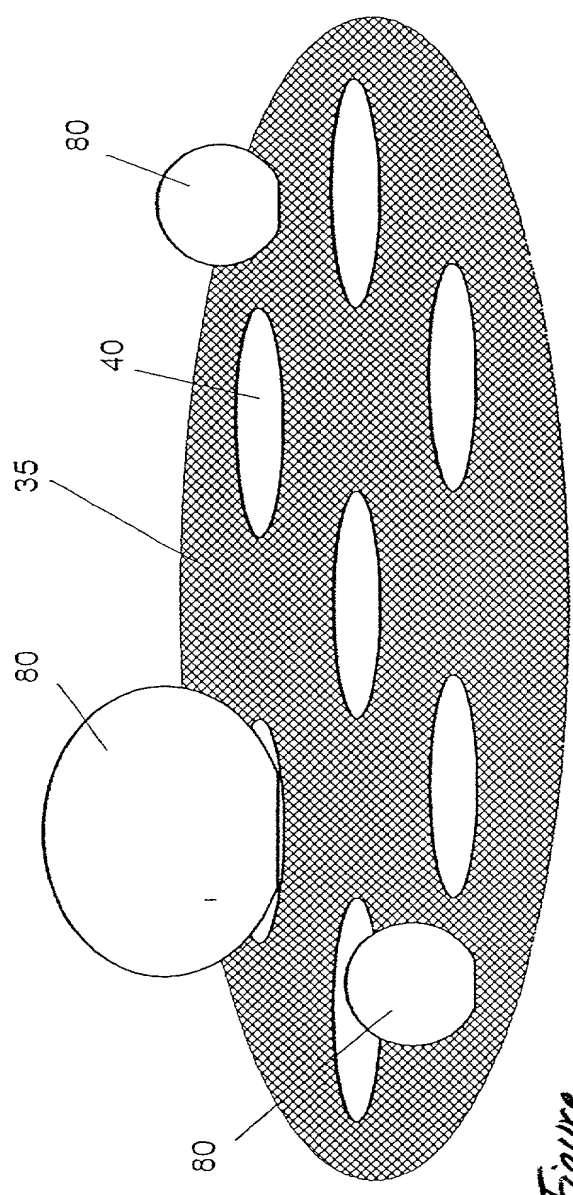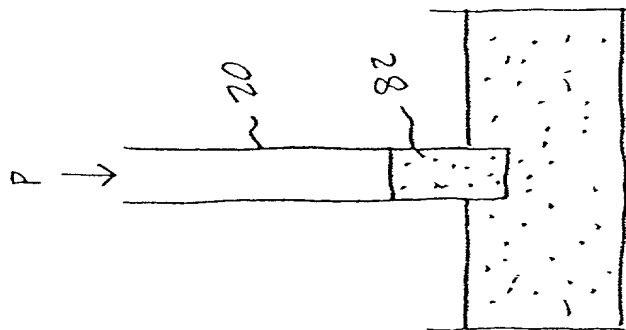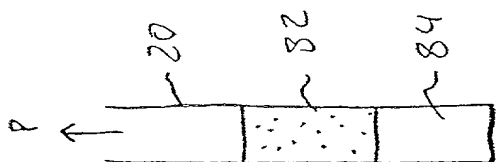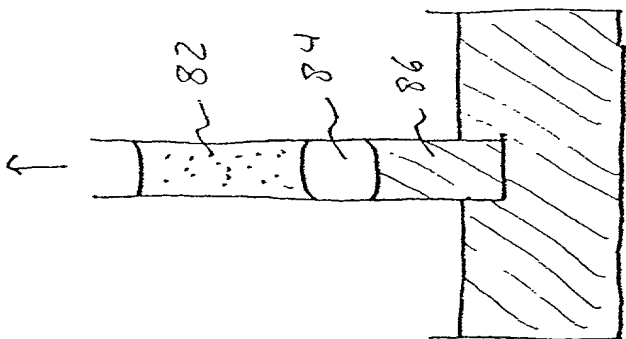
evaporation

40

32

20

30

68

70

74

72

Figure 11

40

30

60

10

20

Figure 12A

80

40

35

80

80

Figure 12B

P →

20

28

A.

P ←

20

82

84

B.

82

84

86

C.

Figure
13

Figure
14A

magnet.

magnetic bead.

Capillary tube.

magnet.

Figure
14B

20

86

85

84

82
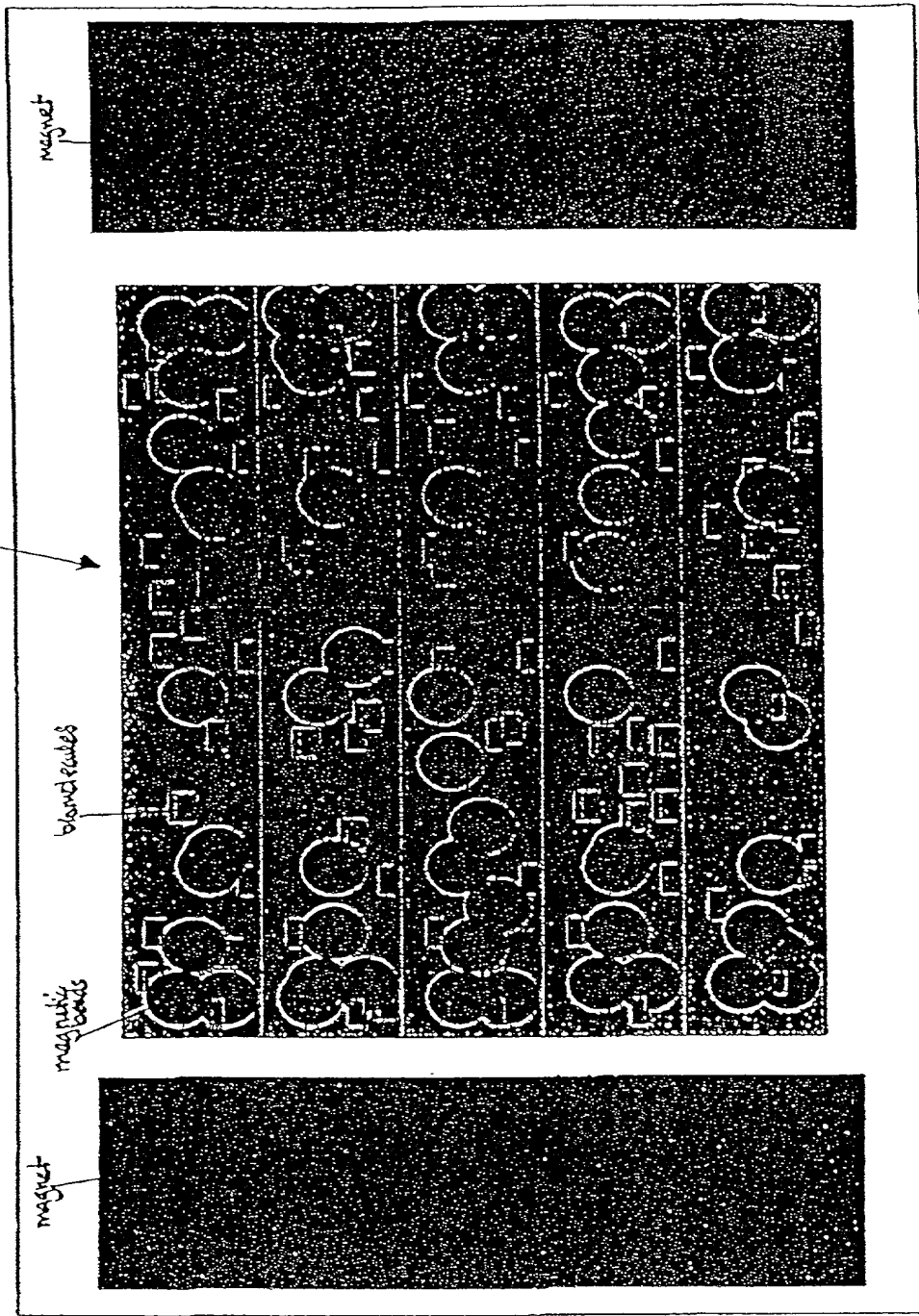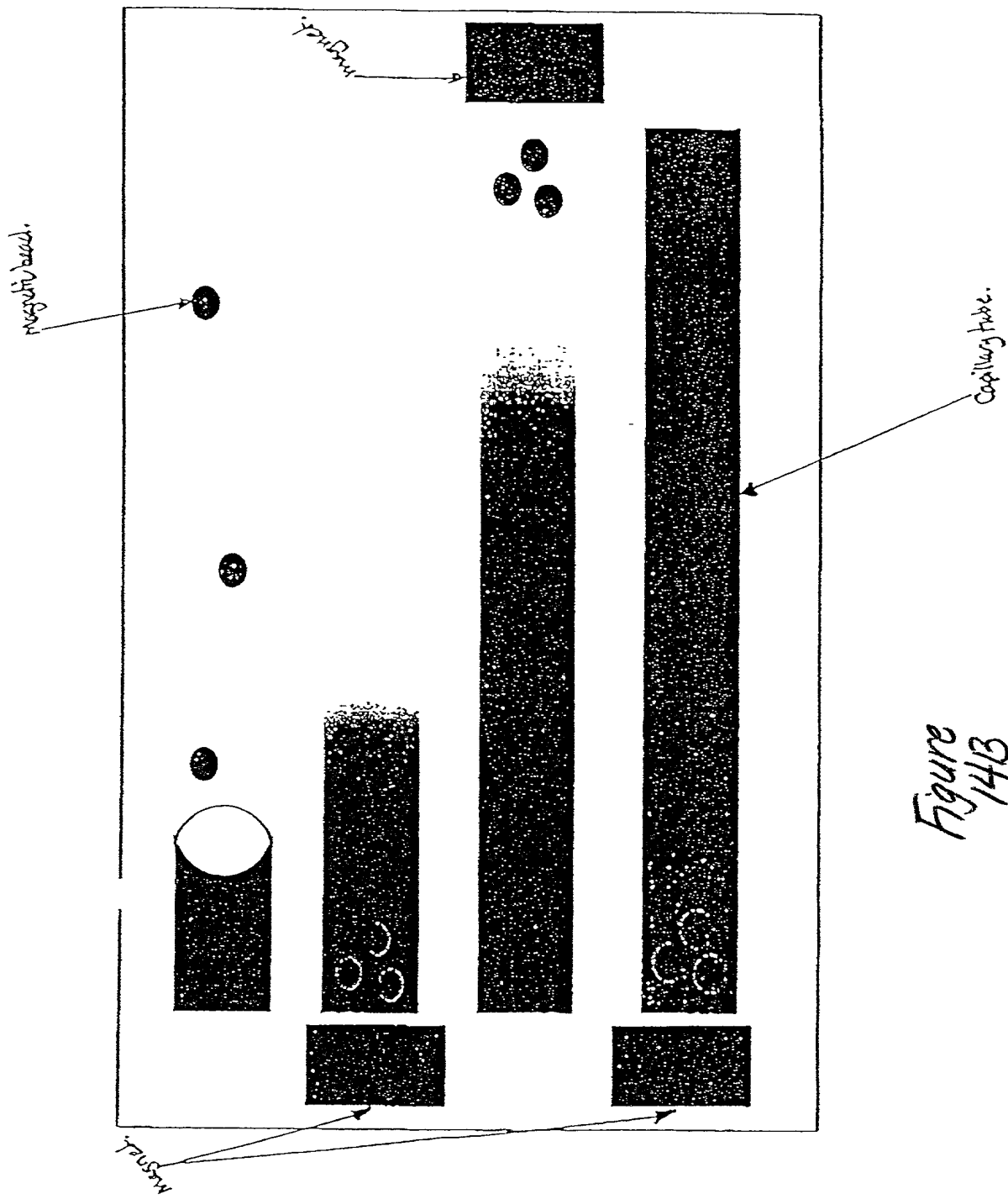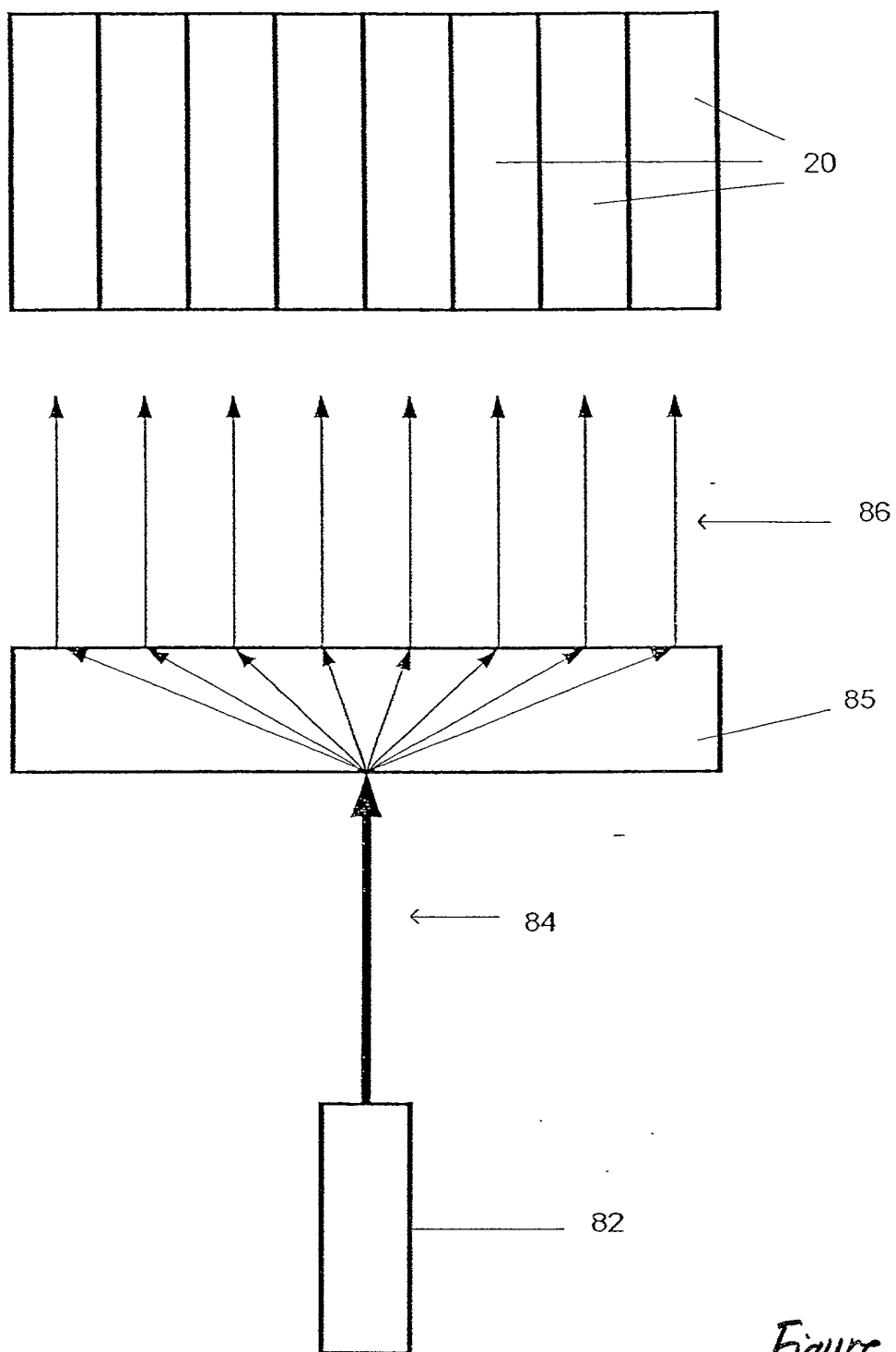
Figure
15

Figure 16

Figure
17

A   B

Cell-retaining filter

Vacuum
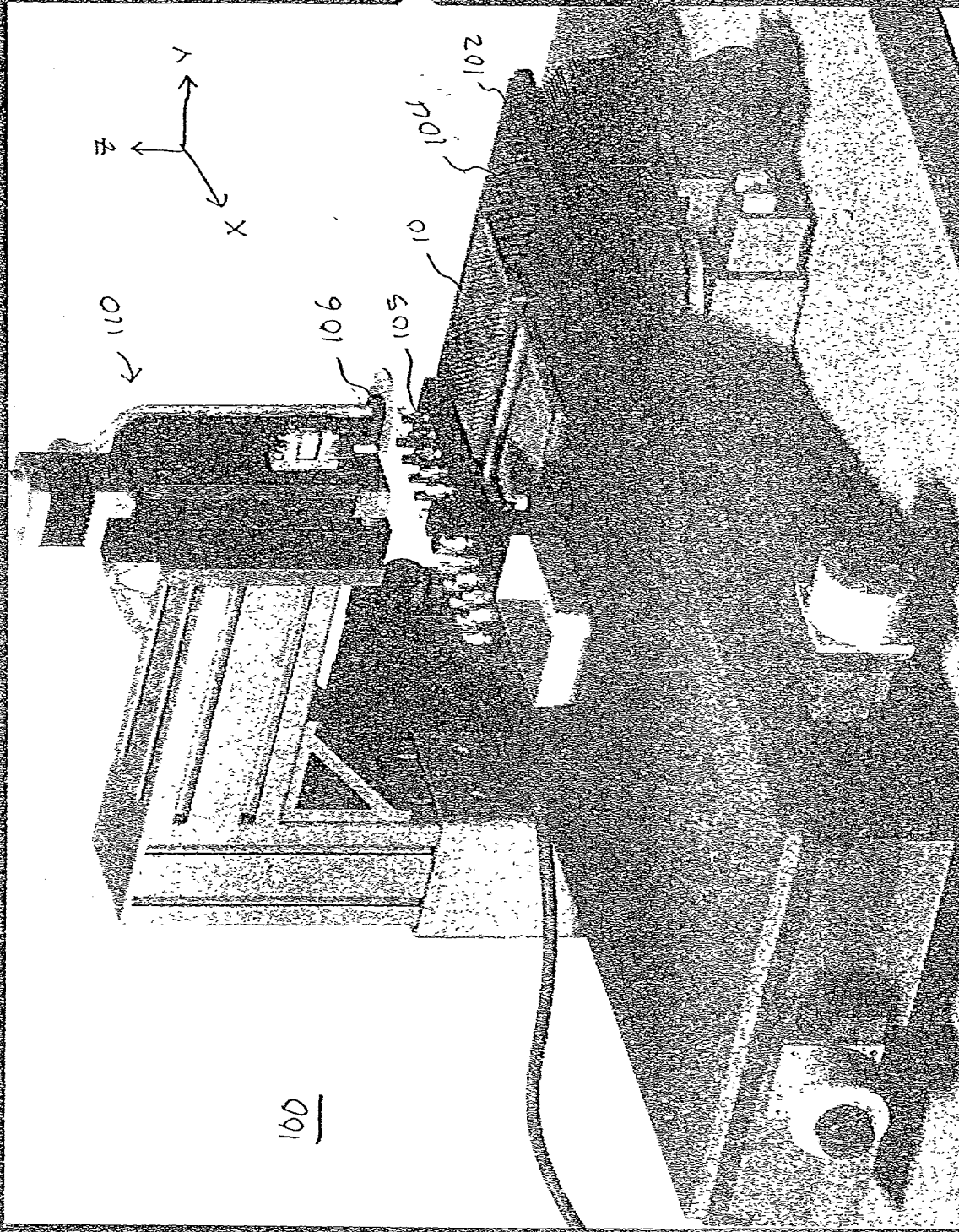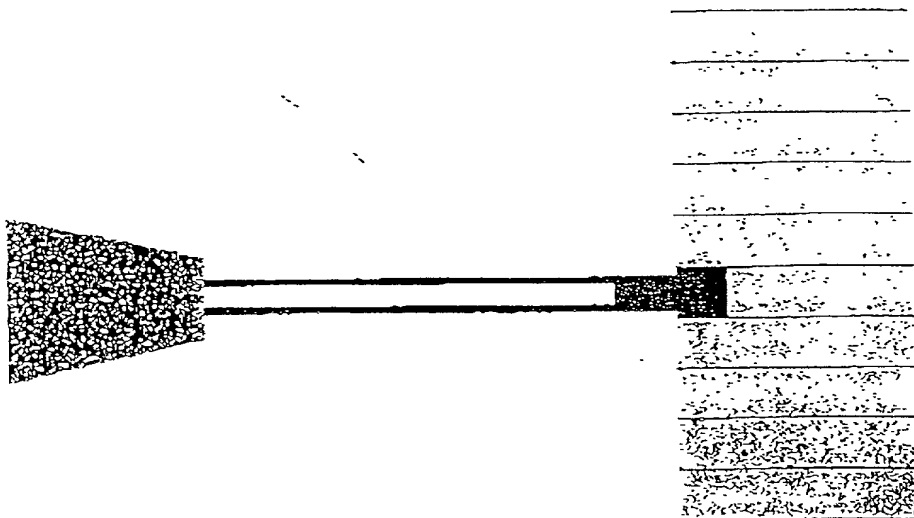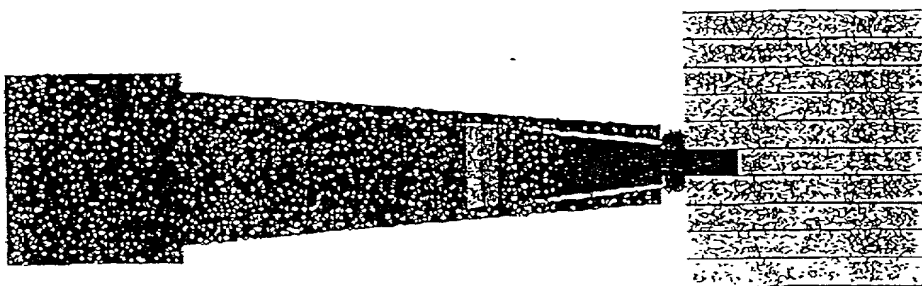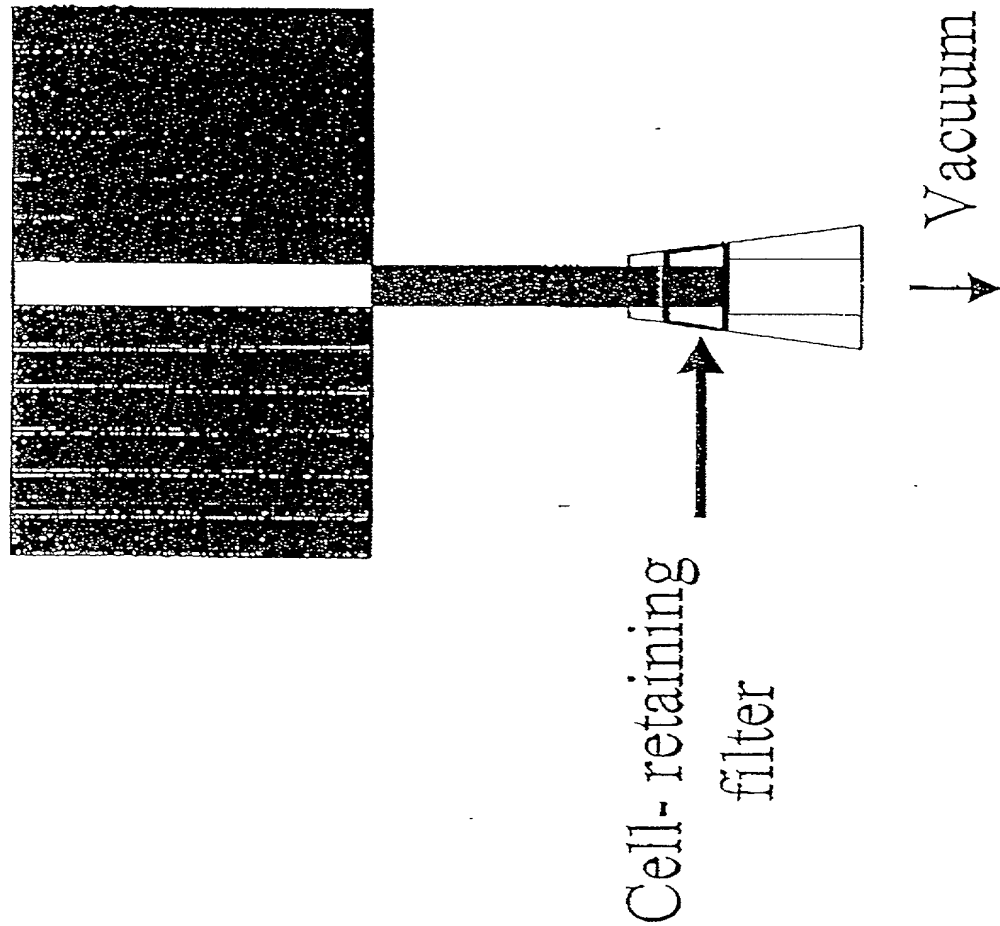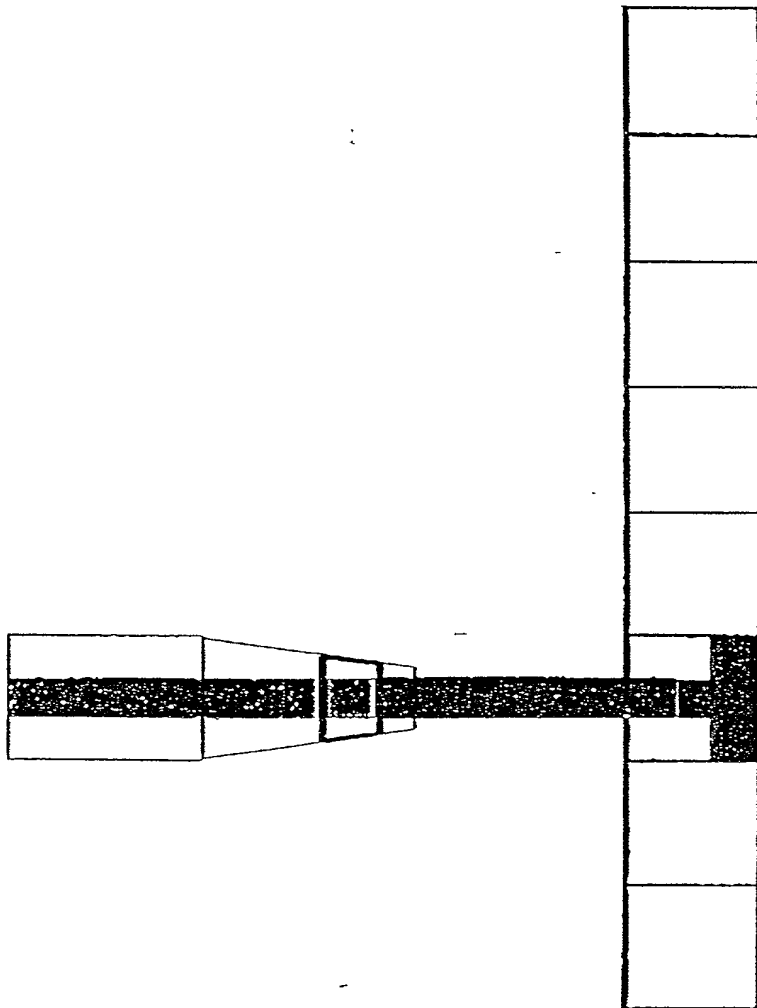
Figure 17c

Figure 17D

HTP Enrichment of Low Copy Gene Targets

E. coli fosmid library

Encapsulate

Grow

Clonal population

Digest Protein

Denature Neutralize

Hybridize, wash

Amplify

Sort for Fluorescence

Transform

FIG. 18

FACS-Biopanning

Clonal population → Digest protein → Sort for fluorescence

EtBr stained DNA

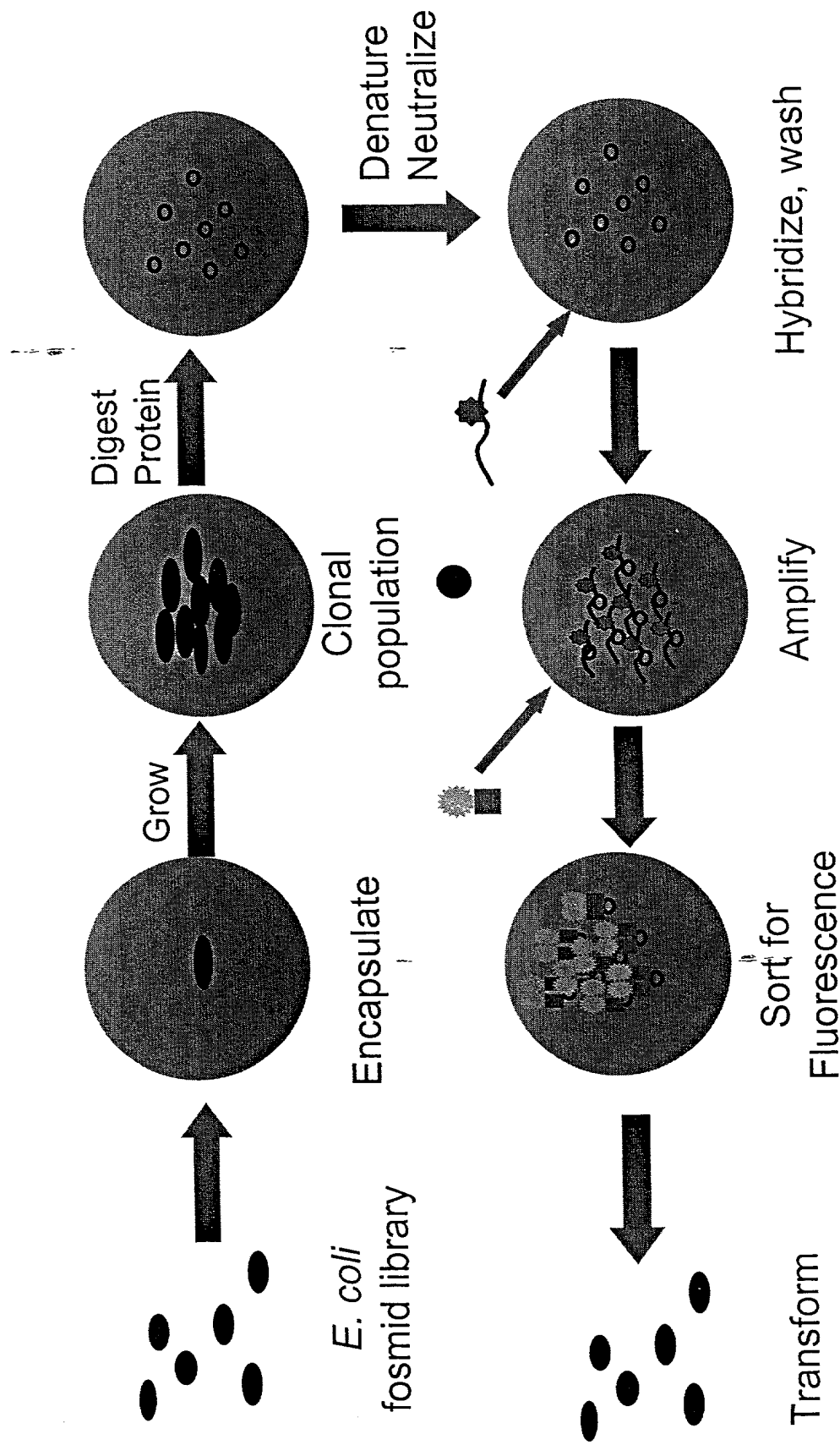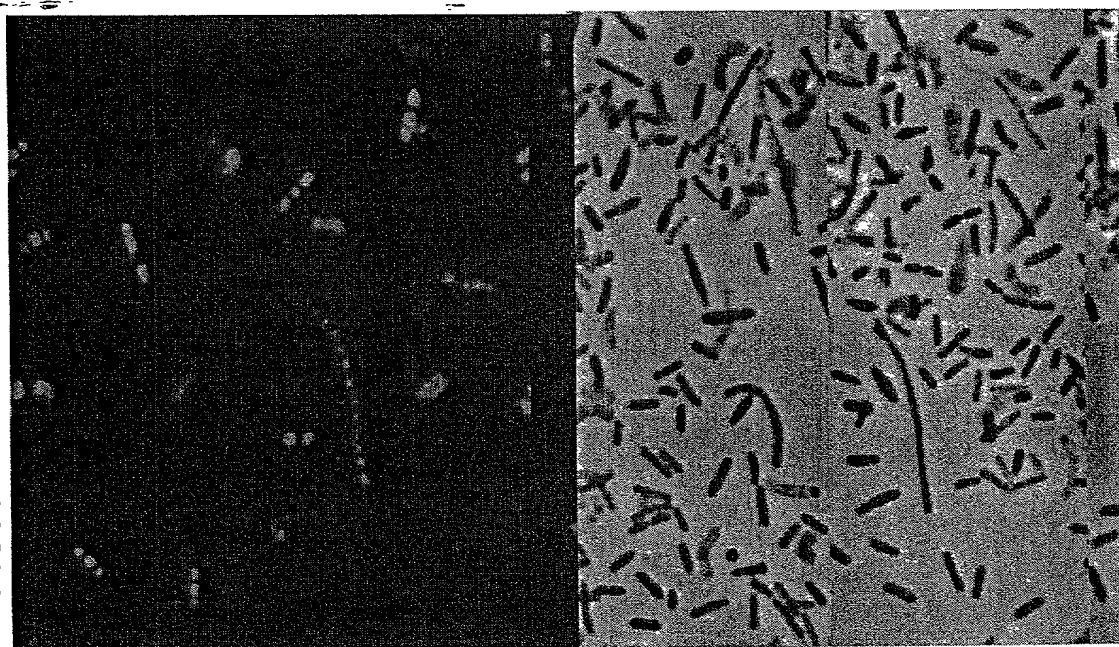Alignement of Environmental PKS Sequence

FIG. 19

Whole Cell Hybridization

Library 557 + 95ES11 clone
Probe: Fluorescein-95ES11

Fluorescein
Excitation

White
light

FIG. 20

FIG. 21

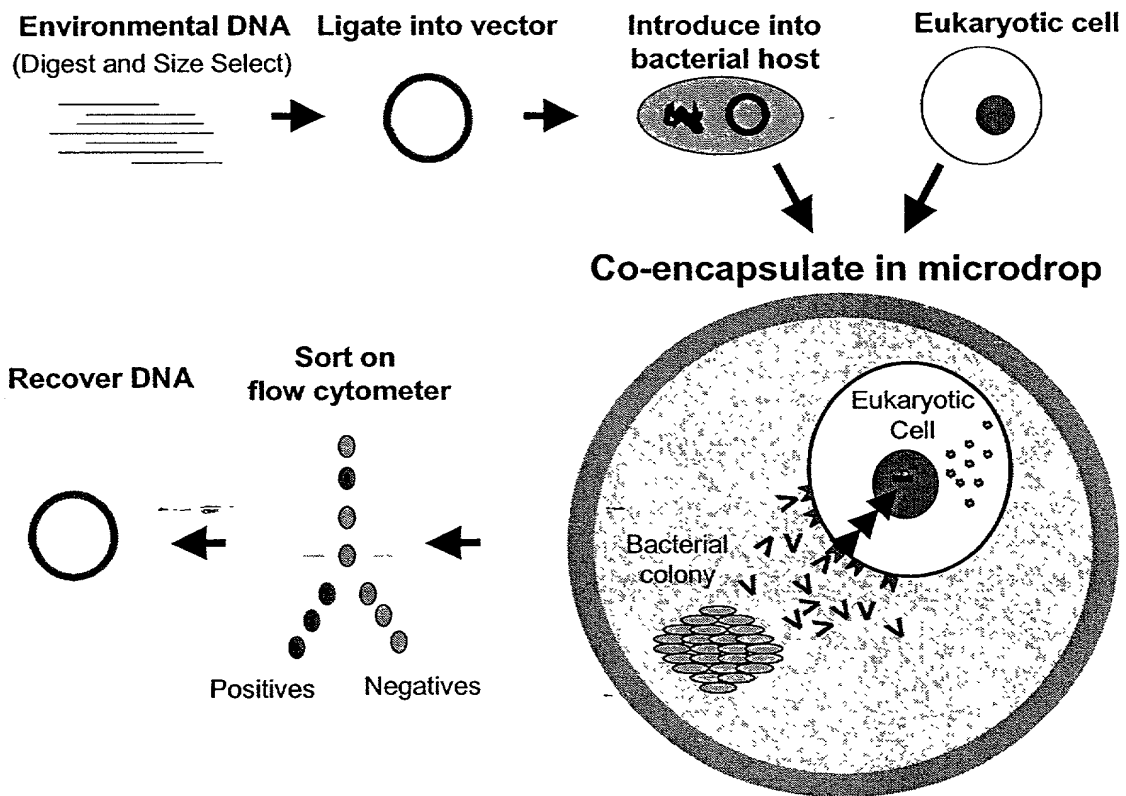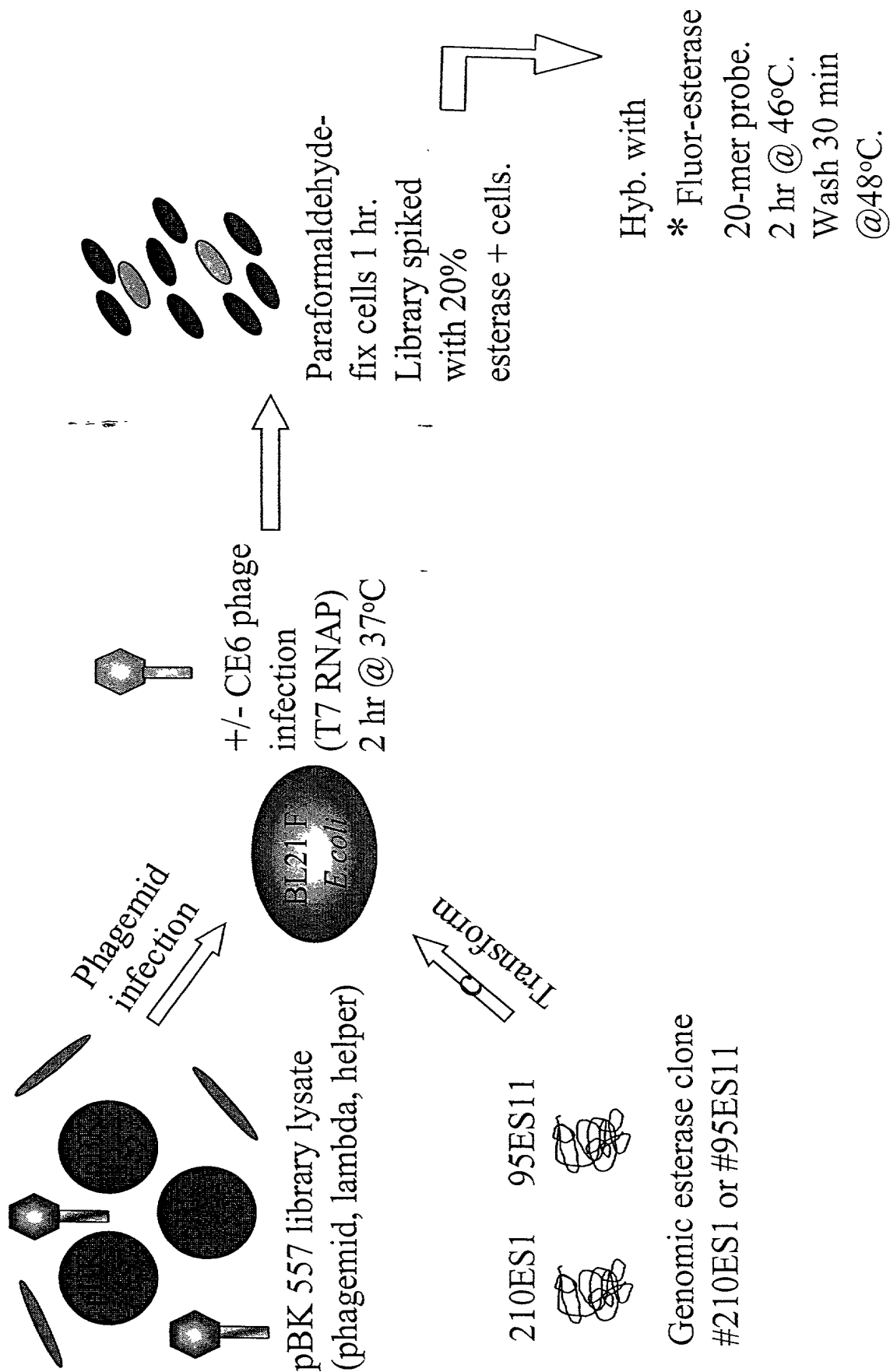# Whole Cell Hybridization Protocol
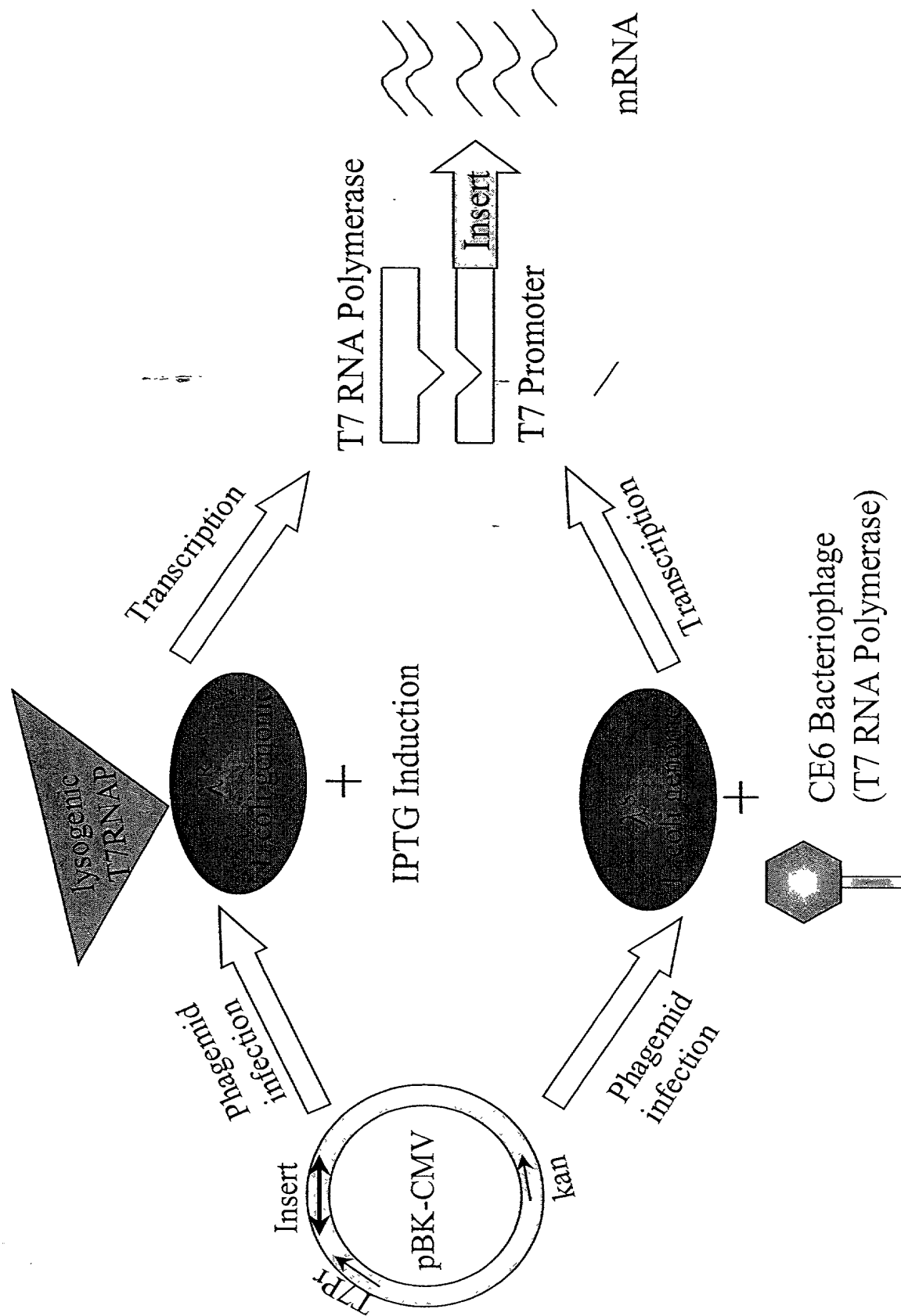

FIG. 22

T7 RNA Polymerase Expression System

FIG. 23